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(54) Title: PATHOGEN-SPECIFIC CTL THERAPY

(57) Abstract

Disclosed is a method for the treatment of a patient infected with an intracellular pathogen, involving administering to the patient an intracellular pathogen-specific, cytotoxic T lymphocyte-stimulatory antigen, or a preparation of the patient's lymphocytes enriched for intracellular pathogen-specific cytotoxic T lymphocytes.

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PATHOGEN-SPECIFIC CTL THERAPY

BACKGROUND OF THE INVENTION

This invention relates to therapies involving administration of cytotoxic T lymphocytes.

Cytotoxic T cells (CTL's) that specifically lyse HIV-1 infected autologous target cells have been found to occur at uncommonly high frequency in the blood of HIV-infected individuals; killing by such cells is predominantly mediated by $CD3^+CD8^+$ effector cells although cytotoxic $CD4^+$ cells and natural killer cells also play a role (Walker et al., *Nature* 328:345, 1987; Plata et al., *Nature* 328:348, 1987; Walker et al., *Science* 240:64, 1988; Sethi et al., *Nature* 335:178, 1988; Koenig et al., *Proc. Natl. Acad. Sci. USA* 85:8638, 1988; Nixon et al., *Nature* 336:484, 1988; Tsubota et al., *J. Exp. Med.* 619:1421, 1989; Riviere et al., *J. Virol.* 63:2270, 1989; Koup et al., *Blood* 73:1909, 1989; Hoffenbach et al., *J. Immunol.* 142:452, 1989; Culmann et al., *Eur. J. Immunol.* 19:2383, 1989; and Hosmalin et al., *Proc. Natl. Acad. Sci. USA* 87:2344, 1990). $CD8^+$ T cells recognize antigenic peptides presented by MHC class I molecules. To be recognized by a CTL, a peptide must be properly processed, be capable of binding to MHC strongly enough to compete with other peptides, and be recognized as a peptide-MHC complex by T cells in the repertoire. Recent studies indicate that in some infections only a small number of peptides meet these criteria and that CTL specific for these epitopes dominate the lytic response (Braciale et al., *Immunol. Rev.* 98:95, 1987; Whitton et al., *J. Virol.* 62:687, 1988; Klavinskis et al., *J. Virol.* 63:4311, 1989; Whitton et al., *J. Virol.* 62:687, 1988;

Braciale et al., *Proc. Natl. Acad. Sci. USA* 86:277, 1989; Townsend et al., *Cell* 44:959, 1986).

Hosmalin et al. (1990, supra) and Takahashi et al. (*Proc. Natl. Acad. Sci. USA* 85:3105, 1988; *Science* 246:118, 1989; *J. Exp. Med.* 170:2023, 1989) report that a small number of epitopes likely dominates the CTL response to HIV-1 in mice. Yamamoto et al. (*J. Immunol.* 144:3385, 1990) and Miller et al. (Abstract FA 74, *Sixth International Conference on AIDS*, San Francisco, CA, 1990) report that in SIV-infected macaques, the CTL response to gag appears to have a limited epitope specificity. Culmann et al. (1989, supra) report that, in two human subjects, the CTL response to HIV-encoded nef protein is predominantly directed against the same 16 amino acid region. Koenig et al. (*J. Immunol.* 145:127, 1990) report that a ten amino acid fragment of the nef protein is recognized by the CTL's of two out of ten HIV-seropositive individuals.

SUMMARY OF THE INVENTION

In one aspect, the invention features a method of treating a mammal infected with an intracellular pathogen involving selecting, from a sample of the mammal's lymphocytes, a sub-sample which is enriched for cytotoxic T lymphocytes which recognize a pathogen-specific antigen and which are capable of lysing pathogen-infected cells of the patient, and administering to the patient a therapeutically effective amount of the sub-sample of cytotoxic T lymphocytes. Preparation of such a pathogen-specific CTL-enriched sub-sample generally involves stimulating the proliferation of a mammal's T-lymphocytes in vitro, using a nonspecific mitogen, in the presence of pathogen-infected cells (i.e., cells displaying pathogen-specific antigens on their surfaces); inclusion of such pathogen-infected cells results in the selective expansion of a population of CTL's which are capable of

targeting and lysing host cells harboring the pathogen. Preparation of the pathogen-specific CTL-enriched sub-sample may further involve identifying, for a particular mammal, those pathogenic-specific antigens which are capable of eliciting a particularly potent CTL response and presenting peptides displaying these antigens to the proliferating T cells in order to further expand the pathogen-specific CTL population in the sub-sample.

In preferred embodiments, the sub-sample is prepared by incubating the sample with a mitogen which is capable of inducing lymphocyte proliferation (preferably, phytohemagglutinin); the sample is further incubated with IL-2; the sample is further contacted with a pathogen-specific antigen recognized by CTL's of the mammal and which is capable of inducing a CTL response in the mammal; the method further involves administering to the mammal a therapeutically effective amount of a pathogen-specific antigen recognized by the CTL's of the mammal and which is capable of stimulating a cytotoxic T lymphocyte response in the mammal.

In another aspect, the invention features a method of treating a mammal infected with an intracellular pathogen involving administering to the mammal a therapeutically effective amount of a pathogen-specific antigen which is recognized by the CTL's of the mammal and which is capable of stimulating a cytotoxic T lymphocyte response in the mammal.

In preferred embodiments of both aspects, the antigen is displayed on the surface of an autologous antigen-presenting cell (preferably a B-lymphocyte); the mammal is a human; the intracellular pathogen is a virus (preferably a human immunodeficiency virus, a human T cell leukemia virus, or a Herpes virus, preferably, an Epstein-Barr virus), a mycobacterium, a protozoan, or a fungus; and the pathogen-specific antigen

immunodominant. In the case of a patient infected with a human immunodeficiency virus, the pathogen-specific antigen is preferably an HIV-encoded protein (for example, the product of the env or the pol gene), or a CTL-stimulatory fragment thereof.

By "intracellular pathogen" is meant a disease-causing organism which resides, during at least a part of its life cycle, within a host cell. By "enriched for cytotoxic T lymphocytes" is meant that the sub-sample has a substantially greater number of pathogen-specific cytotoxic T lymphocytes (i.e., T lymphocytes which recognize and destroy cells bearing foreign antigens, in this case, pathogen-specific antigens, on their surfaces) than a freshly isolated sample of the patient's lymphocytes. By "pathogen-specific antigen" is meant a protein produced upon infection by a pathogen which is recognized (i.e., responded to) as foreign by cells, in this case, cytotoxic T cells, of the patient's immune system. By "lyse" is meant to destroy or disintegrate, for example, a host cell harboring a pathogen. By "pathogen-infected cells" is meant those host cells harboring a pathogen, either in an active or a latent state. By "mitogen" is meant a substance that stimulates mitosis and, thus, cell proliferation. By "CTL response" is meant the proliferation of CTL's in response to, and specific for, a stimulatory antigen. By "autologous" is meant occurring in the same patient. By "antigen-presenting cell" is meant any cell capable of displaying on its cell surface an antigen, or an immunogenic fragment thereof. By "human immunodeficiency virus" is meant, without limitation, HIV-1 and HIV-2; By "human T cell leukemia virus" is meant, without limitation, HTLV-I and HTLV-II; by "Herpes virus" is meant, without limitation, Herpes simplex type 1 and type 2, Herpes zoster, and cytomegalovirus as well as Epstein-Barr

virus. By "virus" is also meant, without limitation, Papillomavirus, Creutzfeldt-Jakob virus, and feline leukemia virus. By "mycobacterium" is meant, without limitation, Mycobacterium leprae, Mycobacterium tuberculosis. By "protozoan" is meant, without limitation, Toxoplasma gondii, Giardia lamblia, Trypanosoma cruzi, organisms of the genus Leishmania, and organisms of the genus Plasmodium which cause malaria. By fungus is meant, without limitation, Pneumocystis carinii, Candida albicans, and Candida tropicalis. By "CTL-stimulatory fragment" is meant a peptide which is capable of stimulating antigen-specific CTL proliferation. By "immunodominant" is meant (an antigen) capable of eliciting an unusually potent CTL response.

In the method of the present invention, the CTL's of the enriched sub-sample or CTL's expanded in the host following administration of pathogen-specific CTL-stimulatory peptide or peptide-bearing antigen-presenting cells recognize and selectively target pathogen-infected cells. Because such pathogen-infected cells represent a small percentage of the total cell population, this method minimizes side effects, such as immunosuppression, which may result from other forms of therapy such as those which destroy or impair the function of host cells which are either infected or at risk of being infected by the pathogen. Moreover, the pathogen-specific CTL population may be administered to the mammal free of lymphokines, thereby avoiding the vascular-leak syndrome generally associated with lymphokine therapies, at least in humans and mice. Finally, the pathogen-specific CTL population is generated from a mammal's lymphocyte sample. This is an important feature of the invention because antigens capable of inducing an effective CTL response (i.e., inducing significant CTL proliferation) may vary, and, for example, in the case of HIV-1, do vary

from one individual to the next. By beginning with a mammal's own lymphocytes, it is possible to enrich for CTL's which recognize and lyse cells bearing pathogen-specific antigens which are immunodominant for that particular human or mammal, thereby maximizing a human's or mammal's CTL response to a pathogenic infection.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The drawing will first briefly be described.

DRAWING

Fig. 1 is a diagrammatic representation of a set of truncated HIV-1 envelope and reverse transcriptase proteins, from which can be derived peptides which are useful as candidate HIV-1-specific, CTL-stimulatory antigens.

Fig. 2 is a schematic representation of the HIV-1-specific epitopes recognized as immunodominant by the CTL's of eight independent HIV-1-seropositive individuals.

EXAMPLE

There now follows a description of a method for generating, from a sample of an HIV-infected patient's lymphocytes, a sub-sample enriched in cytotoxic T lymphocytes (CTL) which recognize an HIV-1-specific antigen and which lyse cells displaying this antigen on their cell surfaces. The method generally involves establishing a T cell line (i.e., population) from a sample of the patient's lymphocytes and stimulating proliferation of this T cell line with a nonspecific mitogen. Because HIV-1 infected cells (i.e., cells displaying HIV-1 antigens on their surface) are naturally

included in the sample in infected individuals and because the culture conditions enhance viral replication, the final preparation is enriched for cytotoxic T lymphocytes, which recognize and lyse cells displaying an HIV-1 specific antigen. The method may further involve identifying, for a particular patient, an immunodominant HIV-1 specific antigen(s) which is(are) capable of inducing a CTL response and presenting a peptide(s) displaying this epitope to the proliferating T cell line to further enrich the sub-sample for HIV-1 specific CTL's.

T-Lymphocyte Culture

Heparinized whole blood was obtained from HIV-1 infected patients, i.e., patients seropositive for HIV-1 as assayed by Western blot analysis, and peripheral blood lymphocytes (PBLs) were isolated by Ficoll-Hypaque density centrifugation. Cells were cultured at $5x10^5$/ml in RPMI 1640 supplemented with 15% fetal calf serum (Hazleton), 2mM HEPES, 2mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50μM β-mercaptoethanol, and T cell lines were established by addition of 2 μg/ml PHA-P (Difco) and 200 U/ml rhu IL2 (Cetus). Twice a week cultures were adjusted to $5x10^5$/ml with fresh IL-2-containing media.

Using this method, T cell lines were generated from ten CDC group II, five group III and three CDC group IV individuals. These cell lines grew vigorously for over a month without further stimulation, even from patients with AIDS, although those cells grew less vigorously. After 6 weeks, most cell lines with no further exogenous stimulation had stopped dividing. No T cell line could be generated from one hemophiliac patient with longstanding AIDS and profound immunodeficiency (absolute CD4 count 20/mm^3, CD4/CD8 ratio 0.03).

After 2-3 weeks of culture, T cell lines were screened for cytotoxicity against autologous B cell lines infected with vaccinia virus containing the HIV-1 env gene or a fragment of the pol gene (i.e., constructs vPE16 and vCF21, respectively, described in Walker et al., 1987, supra; Walker et al., 1988, supra); a B cell line infected with a vaccinia virus containing the lacZ gene was used as a control (i.e., construct vSC8, described in Chakrabarti et al., *Nature* 320:535, 1986; Flexner et al., *Virol.* 166:339, 1988). Autologous EBV-transformed B cell lines were generated from the patient's peripheral blood lymphocyte samples by standard techniques using B95-8 marmoset cell line supernatant. Env-vaccinia (vPE16)-infected cells expressed the gp120 and gp160 (env) proteins from isolate HIV-1_{IIIB}; pol-vaccinia (vCF21)-infected cells expressed all but the last 22 amino acids of the reverse transcriptase (RT) protein from isolate HIV-$1_{HXB.2}$. Expression of HIV-1 proteins was verified by radioimmunoprecipitation of infected cell lines with αHIV serum as described in Essex et al. (Science 220, 859, 1983). Vaccinia virus was prepared and titered by plaque forming assay on CV-1 cells as described in Mackett, M., et al. ("The construction and characterization of vaccinia virus recombinants expressing foreign genes", in D. Glover, ed., DNA cloning: A practical approach, Vol II, IRL Press, Oxford, 1985). Env-vaccinia infected cells were, in some cases, also titered by assaying syncytia formation upon cocultivation with C8166 cells (as described in Salahuddin et al., Virology 129, 51, 1983). For vaccinia virus infection, 1-4 pfu/cell of virus was added to $5x10^5$ exponentially growing B cells in 500 ul of media in a 24 well microtiter plate. Cells were incubated, with rocking, at 37°C over CO_2 for 30 min; an additional 1 ml of media was added to each well, and

cells were further incubated, without rocking, for 16 hr.

Cytotoxicity was measured using a ^{51}-Chromium (Cr) release assay as follows. B cells (i.e., target cells) were pelleted and resuspended in 200ul of serum-containing media to which was added 100uCi of $Na_2(^{51}CrO_4)$ (Dupont). After incubation for 1 hr at 37°C over CO_2 with occasional mixing, targets were washed 3 times and resuspended at 10^5 cells/ml. 10^4 labelled targets were added to triplicate wells of U bottom microtiter plates. For peptide experiments, the labelled targets were incubated with peptide at a final concentration of 50 μg/ml for 30 min. at 37°C over CO_2 before adding effector cells. Effector cells were suspended at various E:T ratios in 100ul of media and added to target cells; plates were incubated at 37°C over CO_2 for 4 hr. For each target, spontaneous release (SR) was determined from wells to which 100 ul of media was added, and total release (TR) was calculated from wells containing 100ul 1% NP40. Supernatants (75ul) were collected from each well and were counted on a gamma counter after addition of 75ul of 1% NP40. Percent specific cytotoxicity was calculated from the average cpm as [(average cpm - SR)/(TR - SR)] x 100. Spontaneous release below 15% of total release was considered acceptable.

Using such an assay, it was demonstrated that 12 out of 18 PHA-stimulated T cell lines directed significant cytolysis of either RT or env-expressing targets (defined as specific lysis of HIV-1 expressing targets minus lysis of the lacZ expressing target of >10%, at E:T of 25:1). Five of the ten group II c lines lysed RT-bearing targets, four lysed env-bearing targets; two of the five group III cell lines lysed RT and env-targets and of the three group IV cell lines, two lysed RT and all lysed env targets. In addition, it was

demonstrated that such T cell lines directed as much as 40% HIV-1-specific cell lysis above background (i.e., lacZ target) lysis in a 4 hr assay using an effector:target (E:T) ratio of 6:1. This was in contrast to autologous, freshly isolated peripheral blood lymphocytes which, from some patients, showed little or no HIV-specific lysis.

Prior to enhanced HIV-1-specific killing, the PHA-stimulated T cell lines acquired NK-like activity, presumably in response to the supraphysiological concentration of IL-2; this effect declined over time in culture.

CTL immunodominant peptides

To selectively enhance the proliferation of HIV-1-specific CTL clones, the cultured T cell line was further stimulated with a peptide which included an immunodominant HIV-1-specific epitope recognized by the patient's CTL.

To identify such immunodominant epitopes for a particular patient, his/her established T cell line was screened after 2-5 weeks in culture for cytotoxicity against autologous B cell targets infected with the vaccinia virus constructs encoding nested truncations of env and RT proteins; cytotoxicity was assayed by ^{51}Cr-release. The epitope was further defined by assaying cytotoxicity (again, by ^{51}Cr-release) against autologous targets which presented short overlapping peptides spanning the region identified as immunodominant using truncated proteins. A representative set of vaccinia vectors expressing nested truncations of the above env and RT isolates (i.e., for env, vPE16, vPE17, vPE18, vPE8, vPE20, vPE21, vPE22; for RT, vCF32-vCF37, described in Hosmalin et al., 1990, supra) are shown in Fig. 1.

Fig. 2 shows that cell lines established from the PBLs of eight independent patients recognize different

immunodominant env and RT epitopes. In particular, in four cell lines studied, response to a single 104 amino acid region (i.e., amino acids 747 to 851) of the env glycoprotein unequivocally dominated cytolysis; in one cell line, an additional region (i.e., 1 to 204) was also recognized. To further refine the commonly recognized 104 amino acid epitope, a set of seven 22-amino acid peptides with 8-amino acid overlaps spanning this region was synthesized by standard techniques (by scientists other than the named inventors) and used to sensitize autologous ^{51}Cr-labelled targets. Three of the four T cell lines that recognized determinants in this region responded to different peptide epitopes. For one subject, a peptide concentration of 0.2 μg/ml was needed to begin to sensitize targets and the response plateaued at 12 μg/ml. For this subject, three separate T cell lines generated over a period of six months consistently recognized the same single dominant epitope. The T cell line from another subject, analyzed with a set of 20-amino acid peptides with 10-amino acid overlaps (obtained from the MRC AIDS Reagent Project), also recognized a single peptide (i.e., amino acids 219 to 238).

Because the HIV-1 isolate used as a source of candidate CTL-stimulatory antigens is likely different from the HIV-1 isolates harbored by the patient and because at least some HIV-1 proteins vary considerably in amino acid sequence between isolates, it is likely that viral antigens identified by this method, e.g., those described above, are relatively invariant.

Following identification of immunodominant epitopes, peptides bearing these epitopes were used to stimulate CTL proliferation as follows. T cell lines (approximately 3-4 weeks after initiation of culture) were harvested and resuspended in fresh IL-2-containing media at 10^6 cells/ml. Cell lines were selected with

either peptide alone (by adding an equal volume of peptide-containing media to a final concentration of 1-50 μg/ml) or peptide presented by autologous antigen-presenting B cells. For the latter, B cell lines were irradiated (5000 rad), resuspended at 10^6/ml and incubated with peptide at 1-50 μg/ml at 37°C with occasional mixing. After 2 hr, the incubated cells were pelleted and added to the T cell line in an equal volume of fresh media. Twice weekly, T cells were counted and fresh media added to maintain a cellular concentration of $5x10^5$/ml. After 10 days, treated T cell lines were tested for cytotoxicity against vaccinia-infected targets.

In one specific example, T cell line 132, derived from a patient with generalized lymphadenopathy, was shown to recognize both an N-terminal and a C-terminal epitope in env but none in RT. To selectively enhance the growth of HIV-1-specific CTL clones with peptide, a three week T cell line from this individual was incubated either directly with the C-terminal env peptide that it recognized (i.e., amino acids 802-823 of sequence, YWWNLLQYWSQELKNSAVNLLN) or with irradiated autologous B cells preincubated with this peptide and washed to remove unbound peptide. In both instances, there was substantial enhancement of HIV-1 specific cytotoxicity to the extent that the killing curves for the peptide-selected cell lines resembled those obtainable from env-specific clones. Peptide concentrations of 1 μg/ml (350 nM) and 50 μg/ml (1.75 uM) were equally effective; the lower concentration fell on the linear part of the peptide dose response curve for sensitizing targets for cytotoxicity; the higher concentration fell on the plateau. The threshold for a response was 0.2 μg/ml or 70nM. Cell lines stimulated with peptide grew less well than untreated cells, possibly because of lysis of

peptide-presenting T cells; the decline in cell numbers was greater at the higher peptide concentration. Cell lines treated with peptide-presenting cells experienced a dramatic burst of cell growth and increased in number 8-fold above untreated cells. They also developed cytotoxicity against EBV-transformed autologous B cells.

Therapy

Sub-samples enriched for pathogen-specific CTL's are administered to a pathogen-infected patient as follows. Cells are washed with PBS to remove culture medium and are infused back into the patient by the standard techniques developed for cancer therapy by Rosenberg (see, e.g., Rosenberg et al., *N. Eng. J. Med.* 319:1676, 1988). Typically, infusion is performed intravenously using 10^9 - 10^{11} cells, and the procedure takes approximately 30 minutes. If necessary, treatment can be repeated. Therapy can be administered soon after pathogen infection or upon onset of symptoms. In addition, one or more PBL samples isolated from a pathogen-infected, asymptomatic individual, or a CTL-enriched sub-sample prepared following pathogen infection, may be stored, frozen in liquid nitrogen, until such time as that patient requires therapy.

Because the CTL's of the enriched sub-sample recognize and selectively target pathogen-infected cells and because such pathogen-infected cells represent a small percentage of the total cell population, this method minimizes side effects resulting from generalized cell damage. In the specific example of an HIV-infected patient, the enriched CTL sub-sample would target HIV-infected CD4 lymphocytes, monocytes and macrophages, leaving other cells of the immune system (including uninfected CD4-bearing lymphocytic and monocytic cells) intact and thus reducing the risk of immunosuppression.

This method also avoids the side effects, e.g., the vascular-leak syndrome associated with lymphokine therapy.

CTL-stimulatory pathogen-specific antigens (e.g., peptides including immunodominant epitopes) are administered in therapeutic amounts to a pathogen-infected patient either as a purified peptide or as a processed antigen presented on the surface of an irradiated autologous antigen-presenting cell (e.g., irradiated autologous B cells or autologous PBLs, incubated with peptide antigen as described in the above example). Because such CTL-stimulatory pathogen-specific antigens expand CTL populations which recognize and selectively lyse pathogen-infected cells, this method minimizes generalized cell damage. Typically, such a peptide antigen would be mixed with a pharmaceutically acceptable carrier (e.g., physiological saline) and administered to a patient by the standard procedures, e.g., intravenous injection. Alternatively, irradiated antigen-presenting cells would be infused back into a patient by the standard techniques of Rosenberg (supra) as described above. Such CTL-stimulatory pathogen-specific antigens may be administered (as described above) at any time following infusion with a pathogen-specific CTL-enriched sub-sample to further stimulate the pathogen-specific CTL response.

When appropriate, lymphokines such as IL-2 or IL-4 may be co-administered with either pathogen-specific CTL-stimulatory peptides or sub-samples of pathogen-specific CTL-enriched lymphocytes to further enhance lymphocyte proliferation. To minimize the side effects often associated with this treatment, a patient may be treated with antihistamines, aspirin or acetaminophen, prior to administration of lymphokines. In addition, a patient may be treated with cyclophosphamide prior to

administration of pathogen-specific CTL-stimulatory peptides or pathogen-specific CTL's.

Pathogen-specific CTL's can combat pathogen infection by recognizing and lysing cells infected with pathogen, thereby preventing further spread of infection. Moreover, certain pathogen-specific CTL's, e.g., CTL's specific for Epstein-Barr virus, can be used to prevent or to treat a virus-induced lymphoma in a patient infected with EBV alone or in a patient infected with EBV and a human immunodeficiency virus.

OTHER EMBODIMENTS

Other embodiments are within the following claims. For example, PBLs may be grown in human serum-containing medium or, alternatively, in serum-free medium (e.g., AIM V, Gibco). Mitogens other than PHA (e.g., concanavalin A, anti-CD3 monoclonal antibody, or anti-T cell receptor monoclonal antibody) and lymphokines other than IL-2 (e.g., IL-4) may be used to stimulate lymphocyte proliferation. Any expression vector capable of transfecting or infecting an antigen-presenting cell may be used in this invention.

CTL-stimulatory antigens may be included in HIV-1-encoded proteins other than env and RT, e.g., they may be included in the gag or nef proteins. To identify such antigens in gag, nef, or in any HIV-1-encoded protein, a complete set of candidate peptides would be prepared by fragmenting HIV-1 cDNA, cloning each fragment into a vaccinia expression vector (or any appropriate expression vector as defined above), and testing for CTL-stimulatory capability as described in the above example. Alternatively, candidate peptides may be synthesized in vitro and tested for CTL-stimulatory activity as described in the above example. An epitope domain could be further refined by expressing sub-fragments of the cloned DNA or by synthesizing sub-fragments of the

candidate peptides, which span the immunogenic region. Large numbers of fragments could be tested simultaneously by attaching candidate peptides to a series of microtiter wells, adding ^{51}Cr-labelled autologous target cells, adding an aliquot of a patient's T cells to each well, and screening for cytotoxicity. Fragments shown to encode a CTL-stimulatory peptide would be administered directly to the patient (as a peptide or presented on the surface of an irradiated, autologous antigen-presenting cell) or would be used to selectively expand an HIV-1-specific CTL population in a sample of a patient's peripheral blood lymphocytes. Any isolate of HIV-1 may be used as a source of candidate viral-specific antigens, and a patient infected with any isolate of HIV-1 (e.g., $HIV\text{-}1_{MN}$) may be treated using the methods of this invention. Proteins or protein fragments homologous to HIV-1-encoded proteins may also be useful in this invention if such proteins or fragments elicit an HIV-1-specific CTL response; such proteins may be coded for by other primate lentiviruses, e.g., HTLV-I and HTLV-II as well as the simian immunodeficiency viruses. T cell lines or pathogen-infected patients may be presented simultaneously with more than one pathogen-specific CTL-stimulatory epitope; such epitopes may be resident in the same or in different proteins.

Similarly, any pathogen-infected mammal (particularly, domesticated animals and livestock) may be treated with a therapeutic amount of a CTL-stimulatory pathogen-specific antigen or with a sub-sample of the mammal's lymphocytes enriched for CTL's which recognize and lyse cells bearing such an antigen, using the methods described above. Moreover, these methods can be used to identify CTL-stimulatory antigens of, and to treat human patients or mammals infected with, other pathogenic viruses including, but not limited to, HIV-2, human T-

cell leukemia viruses, Herpes viruses (e.g., Epstein-Barr virus) as well as any intracellular disease-causing mycobacterium, protozoan, or fungus. Generally, sub-samples of pathogen-specific CTL's would be prepared as described above for HIV-1, i.e., by culturing a patient's lymphocytes in the presence of pathogen-infected cells or in the presence of one or more CTL-stimulatory pathogen-specific epitopes. In the case of an intracellular pathogen which does not reside in peripheral blood lymphocytes, a sample of the patient's or mammal's cells which harbor pathogen would be isolated by standard techniques and co-cultured with a sample of the patient's or mammal's lymphocytes to produce a pathogen-specific CTL-enriched lymphocyte sub-sample.

Pathogen-specific antigens may be presented to T cells in processed form on the surface of an antigen-presenting cell other than a B-lymphocyte, e.g., on the surface of an autologous PBL or an autologous cell of monocytic lineage.

Claims

1. A method of preparing a therapeutic composition comprising taking a sample of lymphocytes from a mammal infected with an intracellular pathogen, and preparing from said sample a sub-sample which is enriched for cytotoxic T lymphocytes which recognize a pathogen-specific antigen and which are capable of lysing pathogen-infected cells of said mammal.

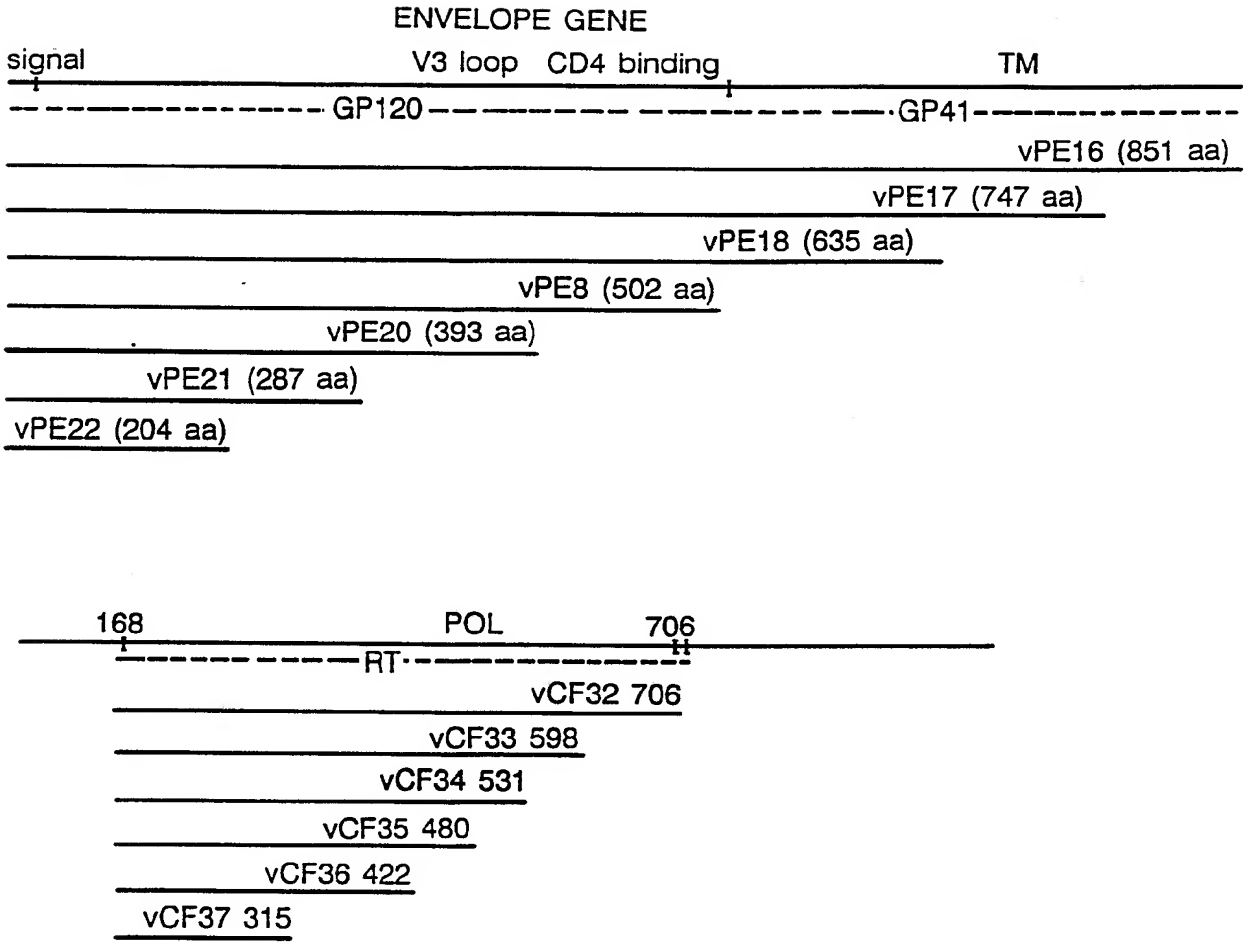
2. A therapeutic composition comprising a preparation enriched for cytotoxic T lymphocytes which are derived from a mammal infected with an intracellular pathogen, which recognize a pathogen-specific antigen and which are capable of lysing pathogen-infected cells of said mammal.

3. A therapeutic composition enriched for cytotoxic T lymphocytes derived from a mammal, said cytotoxic T lymphocytes recognizing a pathogen-specific antigen and being capable of lysing pathogen-infected cells of said mammal, for use in the treatment of said pathogen infected mammal.

4. The use, in the manufacture of a medicament for the treatment of a mammal infected with an intracellular pathogen, of an enriched preparation of cytotoxic T lymphocytes which are derived from said mammal, which recognize an antigen specific for said pathogen and which are capable of lysing pathogen-infected cells of said mammal.

5. The combination of a pathogen-specific antigen and a preparation enriched for cytotoxic T lymphocytes for use in the treatment of a mammal infected with said pathogen intracellularly, said lymphocytes recognizing

said pathogen-specific antigen and being capable of lysing cells of said mammal infected with said pathogen.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GP41
vPE16 (851 aa)
vPE17 (747 aa)
vPE18 (635 aa)
vPE8 (502 aa)
vPE20 (393 aa)
vPE21 (287 aa)
vPE22 (204 aa)
168
POL
706
RT
vCF32 706
vCF33 598
vCF34 531
vCF35 480
vCF36 422
vCF37 315

FIG. 1

IMMUNODOMINANT HIV-1 CTL EPITOPES

Cell Line	MHC Class I	ENV CTL epitope		RT CTL Epitope
		Vaccinia-defined	Peptide	
113	A2,30;B27,w60	vPE16-17	844-863:YRAIRHIPRRIRQGLERILL	vCF34-35
119	A28,--;B17,35	Insufficient lysis		vCF33-34
120	A2,--; B15,18	vPE21-22	219-238: PIPIHYCAPAGFAILKCNNK	vCF35-36
132	A9,31;B5,40	vPE16-17	802-823: YWWWLLQYWSQELKNSAVNLLN	Insufficient lysis
		vpe22	NOT DONE	
136	A2,29;B7,27	vPE20-21	NOT DONE	Insufficient lysis
138	A2,--;B5,27	vPE16-17	788-809: IVELLGRRGWEALKYWWNLLQY	Insufficient lysis
139	A30,w34;B12[44]	vPE20-21	NOT DONE	vCF36-37
142	NOT DONE	vPE16-17	844-863: YRAIRHIPRRIRQGLERILL	Insufficient lysis
		vPE17-18	NOT DONE	

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/06441

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12P 21/02, 21/04; C12N 1/00

U.S. Cl.: 435/70.1,71.1,243

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/70.1,71.1, 243

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Databass: Dialog (Files 5,399,357,72,73,154,155) USPTO Automated Patent System (File USPAT, 1971-1991)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Cell, Volume 44, issued 28 March 1986, Townsend, et al. "The Epitiopes of Influenza Nucleoprotein Recognized by Cytotoxic T Lymphocytes Can be Defined with Short Synthetic Peptides", pages 959-968, see page 966.	1-5
X	Journal of Experimental Medicine, volume 165, issued February 1987, Watari, et al. "A Synthetic Peptide Induces Long-term Protection From Lethal Infection with Herpes Simplex Virus 2" pages 459-470, see page 460.	1-5

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20 NOVEMBER 1991	24 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Lynette F. Smith

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Proceedingsof the National Academy of Sciences, Volume 85. issued May 1988. Takahashi, et al. "An Immunodominant Epitope of the Human Immunodeficiency Virus envelope Glycoprotein gp160 recognized by class I Major Histocompatibility Complex Molecule-Restricted Murine Cytotoxic T Lymphocytes," pages 3105-3109. see page 3106.	1-5
X	Journal of Experimental Medicine. volume 169. issued April 1989. Tsubota, et al. "cytotoxic T Lymphocyte Inhibits Acquired Immunodeficiency Syndrome Virus Replication In Periperal Blood Lymphocytes", pages 1421-1434, see page 1422.	1-15